Atheroarteriosclerosis Induced by Infection With a Herpesvirus

C. Richard Minick, MD, Catherine G. Fabricant, MS,
Julius Fabricant, VMD, PhD, and Maria M. Litrenta, MD

Atheroarteriosclerosis closely resembling that in humans was induced in normocholesterolemic and hypercholesterolemic chickens by infection with Marek's disease herpesvirus (MDV). Four comparably sized groups of chickens were used. Each group was initially fed a diet relatively poor in cholesterol. Groups I and II were inoculated intratracheally at 2 days of age with MDV. At 15 weeks, one group of virus-infected chickens (Group II) and one group of uninfected controls (Group IV) were fed a 2% cholesterol supplement for an additional 15 weeks. Groups I, infected, and III, uninfected, were continued on a cholesterol-poor diet. All groups were killed at 30 weeks. Striking grossly visible atherosclerotic lesions were seen in large coronary arteries, aortas, and major aortic branches of both Groups I and II but not in those of Groups III and IV. Microscopically, arterial changes in infected animals were characterized by occlusive fibromuscular intimal thickening, which formed fibrous caps overlying areas of atheromatous change. This change closely resembled chronic atherosclerosis in humans. These results may be important to our understanding of human arteriosclerosis, since there is widespread and persistent infection of human populations with as many as five herpesviruses. (Am J Pathol 96:673–706, 1979)

THE ETIOLOGY and pathogenesis of atherosclerosis are poorly understood. Identified risk factors account for only a portion of the high incidence of atherosclerosis observed in the continental United States.[1] Thus, unidentified factors acting either alone or in synergy with recognized risk factors must contribute significantly to this high incidence of atherosclerosis. Although hypercholesterolemia is commonly considered a highly significant risk factor, there is experimental and clinicopathologic evidence to indicate that arterial injury may have a primary role in the pathogenesis of atherosclerosis.[2-16] Arterial injury and the associated local reactive changes may provoke intimal thickening and other reparative changes that favor deposition of blood-borne lipid at sites of injury and lead to atherosclerosis. On the other hand, Benditt and Benditt observed that cells comprising some human atheromas are monotypic.[17] These observations have been confirmed by Pearson et al [18] and have been

From the Department of Pathology, The New York Hospital—Cornell Medical Center, New York, and the Departments of Microbiology and Avian and Aquatic Animal Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

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Address reprint requests to C. Richard Minick, MD, Department of Pathology, The New York Hospital—Cornell Medical Center, 1300 York Avenue, New York, NY 10021.

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recently extended to include cells in organizing thrombi.[19] As a result of finding that cells in some atheromas are monotypic, Benditt suggested that atheromatous plaques may not result from arterial injury but may be analogous to neoplasms and induced by the effect of chemical or viral mutagens on vascular smooth-muscle cells.[20]

Viruses may be important in atherosclerosis as inciters of arterial injury or as mutagens, or they may alter lipid metabolism.[21,22] In spite of these considerations, the role of viruses in the pathogenesis of arteriosclerosis has received little attention. We have recently reported in a short communication that infection with Marek's disease herpesvirus (MDV) will lead to occlusive atherosclerosis in hypercholesterolemic and normocholesterolemic specific-pathogen-free (SPF) chickens.[23,24] The purpose of this communication is to report in detail on the atherosclerosis induced in those experiments.

Materials and Methods

Chickens

A total of 290 chickens were used in these experiments. Chickens were from a strain of SPF White Leghorn chickens maintained by the Department of Avian and Aquatic Animal Medicine at The New York State College of Veterinary Medicine, Cornell University, Ithaca, New York. These SPF chickens are free of Marek's disease and all other known viral and microbial pathogens.* The genetic strain used, ie, P-line, is moderately susceptible to infection with MDV.[25]

Virus Stock

The Marek's disease herpesvirus used in this experiment was CU-2, a cell-free, clone-purified strain of MDV. This strain is of relatively low virulence and produces primarily neural and gonadal tumors in susceptible chickens.[26]

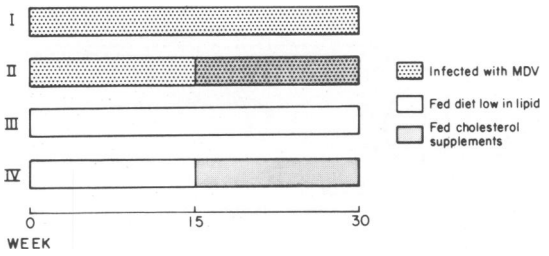
Diets

All chickens were initially fed a commercial diet *ad libitum*. This diet is relatively poor in cholesterol (cholesterol concentration does not exceed 0.025% by weight). Later in the experiment, some chickens were fed *ad libitum* the same diet supplemented with 2% (wt/vol) cholesterol (Cholesterol, U.S.P., ICN Pharmaceuticals, Cleveland, OH).

Collection of Serum and Estimation of Cholesterol Concentrations

Concentrations of total serum cholesterol were determined by a modification of the Lieberman-Burchard method [27] using a Coulter automated analyzer. Unfasted chickens in all groups were bled at Week 15 of the experiment prior to initiation of cholesterol feeding and at approximately 5-week intervals thereafter. Serums were harvested promptly and stored at −20 C until analyzed.

* These chickens are regularly tested for and are free of Newcastle disease, infectious bronchitis, Marek's disease, lymphoid leukosis viruses, infectious bursal disease virus, avian encephalomyelitis, *Salmonella* infections, and *Mycoplasma* infections. Necropsies are performed on all chickens that die in the basic breeding flocks and tissues are cultured when appropriate.



TEXT-FIGURE 1—Design of experiment.

Agar Gel Precipitin Tests

Agar gel precipitin tests for antibody to Marek's herpesvirus were performed on serum collected from all birds at 15 and 30 weeks of age by methods described previously.[28]

Experimental Groups

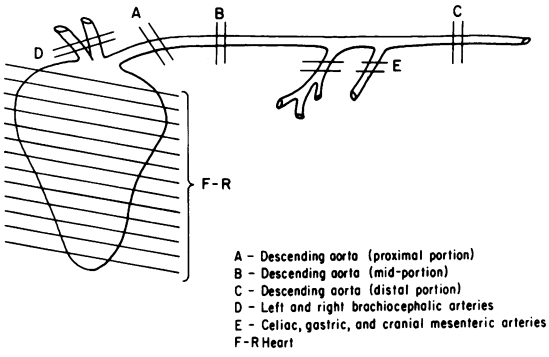
Four comparably sized groups, a total of 290 chickens, were randomly selected from the SPF P-line stock. One hundred sixty chickens were inoculated intratracheally at 2 days of age with 100 focus-forming units of the CU-2 strain of MDV suspended in 0.2 ml of M-2 buffer.† Infected chickens and the remaining uninfected chickens were housed separately in isolation units and initially fed a commercial diet relatively poor in cholesterol. At Week 4, 81 infected and 70 uninfected female chickens were killed. These hens were killed to avoid variations of cholesterol metabolism associated with egg production. Some infected chickens died with manifestations of Marek's disease. (See Results.) The remaining chickens, 130 cockerels, 70 infected and 60 uninfected, were then divided into four experimental groups, and each group was housed separately in isolation units (Text-figure 1). At 15 weeks, one group of 29 virus-inoculated chickens (Group II) and 23 uninoculated controls (Group IV) were fed the same diet supplemented with 2% cholesterol for 15 additional weeks. Twenty-nine chickens of Group I and 28 chickens of Group III were continued on the cholesterol-poor diet.

Autopsy Procedures

Autopsies were not performed on chickens that died or were killed prior to the fifth week of the experiment. Chickens were killed by cervical dislocation. Aortas, brachiocephalic arteries, coronary arteries, and celiac, mesenteric, and gastric arteries were examined *in situ* for grossly visible atherosclerosis.

Brachiocephalic arteries and the entire aorta were opened along their ventral aspect, and their luminal surfaces were examined for evidence of grossly visible atherosclerosis. Comparable numbers of tissue blocks, usually two to three blocks, were taken randomly from each brachiocephalic artery and from the proximal portion, mid-portion, and distal portion of the descending aorta, as well as from the celiac, gastric, and cranial mesenteric arteries (Text-figure 2). After fixation, the heart was sliced at 2–3-mm intervals from apex to base in a plane roughly parallel to the atrioventricular groove in a manner similar to that used by Paterson et al.[29] This resulted in comparable numbers of 2–3-mm tissue blocks, usually 12 to 14 for chickens in each experimental group. Tissue blocks were routinely embedded in paraffin, and 5-μ sections were cut at three levels in each block. All sections were routinely stained with hematoxylin and eosin. Many adjacent sections were also stained with Weigert–van Gieson stain for elastic tissue. In many instances, tissue

† M-2 buffer consists of tissue culture medium 199 supplemented with 10% tryptose phosphate broth buffered to pH 7.2 with sodium bicarbonate.



TEXT-FIGURE 2—Sites of tissue blocks.

blocks adjacent to those embedded in paraffin were embedded in 7.5% gelatin and frozen in liquid nitrogen.[30] Frozen sections from these gelatin-embedded blocks were cut at 3–5 μ, stained with oil red O for fat, and counterstained with hematoxylin.

Microscopic Observations

Sections of hearts, kidneys, livers, aortas, and major aortic branches were examined for microscopic lesions. One section from each of the tissue blocks of heart was examined. Arterial lesions were enumerated and classified according to the size of the artery involved, ie, large, medium, or small, and to the qualitative character of the lesion, ie, fatty, proliferative, or fatty-proliferative.[8] Main arteries and major branches were classified as large; the intramyocardial arteries were classified as medium or small. The extent of coronary arterial lesions was assessed by counting the number of lesions and scoring each according to the degree of luminal occlusion as follows: 1, 0–10% stenosis; 2, 10–70% stenosis; and 3, greater than 70% stenosis. The total score for each chicken heart was obtained by adding the scores for all of the arterial lesions. The extent of the disease was analyzed with regard to the size of the arteries involved and the character of the arterial lesions.

Atherosclerotic change in the aortas, brachiocephalic branches, and celiac, mesenteric, and gastric arteries was classified according to the qualitative character of the lesion: fatty, proliferative, or fatty-proliferative. The degree of arteriosclerotic change was assessed by measuring the thickness of the intima using an ocular micrometer at a magnification of 500 ×. The intima was measured at its thickest point; in all instances, the thickness of the underlying media was also measured. We recognize that evaluating intimal thickness and luminal occlusion in specimens that are not perfusion-fixed is not optimal. However, the number of animals was too large to perfuse and blood vessels of all groups that we compared were fixed in the same manner.

Sections from all portions of the arterial system were examined for the presence of nerve lesions. Nerve lesions in each section were scored according to the degree of lymphocytic infiltration as follows: 0.5, only a few widely scattered lymphocytes; 1, diffuse infiltration with moderate numbers of lymphocytes; and 2, diffuse infiltration with many lymphocytes. The score in all the sections from 1 animal was added to give a total score for nerve lesions in each chicken.

All heart sections from each experimental group were examined for infiltration with lymphocytes and myocardial necrosis. Lymphocytic infiltrate was classified with regard to the size of lymphocytes seen, ie, small and intermediate or large, as well as for the pattern of the infiltrate, ie, a patchy diffuse infiltrate or discrete tumor masses. Myocardial infiltration in each section was quantitated morphometrically and scored as follows: 0.5, 0–4

foci of infiltration; 1, 4–10 foci of infiltration; 2, 10–20 foci of infiltration; 3, 20–30 foci of infiltration; and 4, over 30 foci of infiltration. The score for each section was added to obtain a total score for the heart of each chicken.

Fluorescent Antibody Tests

Frozen sections of brachiocephalic arteries in each animal and some gastric arteries were examined for intracellular Marek's herpesvirus antigen by staining with specific Marek's herpesvirus antibody conjugated with fluorescein isothiocyanate. Techniques for preparation of reagents and testing of specificity of antibody and staining were performed as described.[31]

Statistical Analyses

Mean serum cholesterol concentrations of experimental groups were compared using an independent *t* test. Since the data regarding arterial lesions were often nonparametric, 2 × K contingency tables were used to test the significance of differences between the number of chickens with arterial lesions in each experimental group. Data from the following observations were compared between experimental groups: 1) number of chickens with arterial lesions in various portions of the arterial system; 2) frequency of coronary arterial lesions with respect to distribution among large, medium, or small coronary arteries and character of change; 3) frequency of coronary lesions with respect to extent, ie, number of lesions and degree of luminal occlusion, and aortic lesions with respect to intimal thickness; and 4) frequency of mesenteric artery lesions with respect to extent of luminal occlusion. Correlation of intimal thickness to medial thickness in various portions of aorta was tested using a Spearman rank-order correlation (r_s). Correlation between serum cholesterol concentration and the number of coronary arterial lesions was similarly tested. Since the data were normally distributed, correlation between the total score of nerve lesions or myocardial lesions and the number of coronary arterial lesions was tested using Pearson product-moment correlation (r).

Results

Survival of Chickens

Thirty-nine, or 24%, of the 160 chickens initially infected with MDV died with clinical and pathologic manifestations of Marek's disease. Twenty-one, or 13%, died with manifestations of Marek's disease during the first 15 weeks of the experiment. Of 58 infected cockerels starting the second 15 weeks of the experiment, 18, or 31%, (7 chickens of Group I and 11 chickens of Group II) died with clinical and pathologic manifestations of Marek's disease. In addition, 81 infected hens were killed at Week 4. The 22 chickens of Group I and 18 chickens of Group II that survived the 7-month experimental period were included in the data analyzed for this report. Six Group I chickens and two Group II chickens that survived approximately 6 months were also included, as indicated below. Of the 130 uninfected chickens, 10 died of nonspecific causes unrelated to Marek's disease prior to Week 15 of the experiment. Since these chickens died before the cholesterol supplement was fed to Group IV, they were not included. Seventy control hens were killed at Week 4 of the experi-

ment. Thus, 50 uninfected chickens (27 in Group III and 23 in Group IV) survived for the entire 7 months. Chickens of Groups I and II which survived more than 6 months of the experiment were used for comparison of incidence of chickens with arterial lesions and distribution and type of coronary lesions. Only chickens surviving for the entire experimental period were used for comparison of serum cholesterol concentrations and extent of coronary and aortic arterial lesions.

Serum Cholesterol Concentrations

Prior to feeding the cholesterol-supplemented diet, the concentration of total serum cholesterol was normal for chickens of all groups, and the overall means ranged between 128.5 ± 3.9 mg/100 ml and 154.0 ± 14.2 mg/100 ml (mean ± SEM). As reported by other investigators, total serum cholesterol concentrations for chickens fed a commercial mash diet were within a range of 65–159 mg/100 ml.[32] Serum cholesterol concentrations for all chickens of Groups I and III were within the normal range for the second 15 weeks of the experiment, with overall means of 137.1 ± 6.2 mg/100 ml and 137.8 ± 3.7 mg/100 ml, respectively. Overall mean serum cholesterol concentrations of chickens in Groups II and IV increased to 481.4 ± 44.4 mg/100 ml and 388.1 ± 33.7 mg/100 ml, respectively, during the second 15 weeks of the experiment, when chickens were fed cholesterol-supplemented diets. Mean cholesterol concentrations in Groups II and IV were not significantly different (t test = ns). Concentration of serum cholesterol was significantly correlated with the number of coronary arterial lesions (see below) in chickens of Group IV ($r_s = 0.70$, $P < 0.005$). There was no correlation between coronary lesions and serum cholesterol concentrations in chickens of Group II.

Agar Gel Precipitin Tests

Agar gel precipitin tests for MDV antibody were performed on serums from all birds at 15 and 30 weeks of age. Serums from all uninoculated chickens in Groups III and IV were negative for MDV antibodies. Approximately 80% of serums from chickens in Groups I and II were positive for MDV antibodies. The finding that only 80% of inoculated chickens developed precipitins was not unexpected. Results of previous experiments have indicated that P-line chickens have less than 100% incidence of agar-gel precipitins to MDV although all are infected, as shown by virus neutralization or fluorescent antibody tests.[33,34] Therefore, it is reasonable to infer that all of the inoculated chickens of Groups I and II were infected with MDV. Added evidence of the high level of MDV infection in Groups I and II was the death of approximately 30% of the birds included in the analysis with grossly visible lesions of Marek's disease (see below).

Gross Morphologic Observations

Grossly Visible Arterial Lesions

Grossly visible atherosclerosis was seen in large coronary arteries, aortas, and major aortic branches of 7 of 18 Group II chickens infected with MDV and fed cholesterol supplement. In many portions of the arterial system, this atherosclerotic change was very marked and often nearly occluded the lumens of entire segments of muscular arteries (Figures 1–4, 7, and 13–16). In other areas, plaques were focal and measured only 1 or 2 mm in diameter. Grossly similar but less fatty lesions were seen in arteries of 3 of 22 chickens of Group I infected with the virus but not fed cholesterol supplement. Many of these lesions also occluded major segments of the arterial system (Figures 11 and 12). In contrast, grossly visible atherosclerosis was not seen in these portions of the arterial system in 23 normocholesterolemic chickens of Group III and 27 hypercholesterolemic chickens of Group IV not infected with MDV. Thus, grossly visible atherosclerosis involving aortas, large coronary arteries, and other major aortic branches was seen in 10 of 40 infected chickens and in none of 47 uninfected controls ($\chi_1^2 = 14.06$, $P < 0.001$).

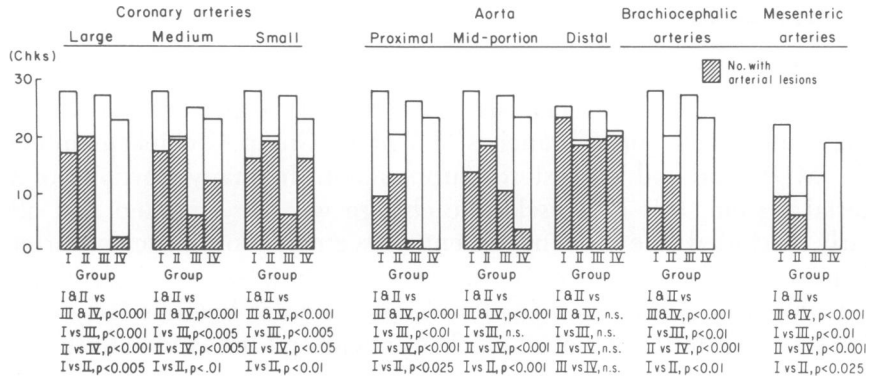
Grossly Visible Marek's Disease Lesions

Fourteen of the 48 infected chickens included in the data analyzed (30%) had grossly visible evidence of Marek's disease. Five of 22 chickens in Group I had peripheral nerve lesions consistent with Marek's disease; 3 chickens had visceral tumors. One chicken had both tumors and nerve involvement. Three of 18 chickens in Group II had evidence of nerve involvement and 2 had visceral tumors. None of the chickens in Groups III or IV had evidence of Marek's disease.

Microscopic Morphologic Observations

Microscopic Arterial Lesions

The number of virus-infected chickens with microscopic lesions in the coronary arteries; gastric, celiac, or mesenteric arteries; and proximal and mid-portions of the descending aorta was significantly greater than the number of uninfected chickens with lesions in these arteries (Text-figure 3). In the coronary arteries, this difference was particularly striking in the large arteries. Thus, 17 of 28 Group I chickens and all of 20 Group II chickens had microscopic lesions of large coronary arteries compared with none of 27 in Group III and 2 of 23 in Group IV ($\chi_1^2 = 20.95, 41.47$; $P < 0.001$). The number of chickens with lesions in both medium and small arteries was significantly greater in infected chickens of Groups I and II



TEXT-FIGURE 3—Number of chickens with microscopic arterial lesions.

compared with those in the corresponding uninfected controls (Groups III and IV) (medium arteries: $\chi_1^2 = 8.37, 9.75$; $P < 0.005$; small arteries: $\chi_1^2 = 9.89, 4.57$; $P < 0.005$, $P < 0.05$). Overall, there was a significantly greater number of chickens with lesions in coronary arteries of all sizes in Group I compared with Group III and Group II compared with Group IV ($\chi_1^2 = 16.64, 8.55$; $P < 0.001$, $P < 0.005$). The number of chickens with lesions in large, medium, and small coronary arteries was significantly greater in Group II compared with Group I ($\chi_1^2 = 10.19, 7.73, 6.6$; $P < 0.005$, $P < 0.01$, $P < 0.01$). Feeding cholesterol-supplemented diets to uninfected chickens (Group IV) resulted in a significant increase in the number of those with arterial lesions in medium and small coronary arteries compared with Group III ($\chi_1^2 = 4.84, 11.30$; $P < 0.05$, $P < 0.001$). The slight increase in the number of uninfected cholesterol-fed chickens with lesions in large coronary arteries was not shown to be significant. Thus, infection with Marek's disease herpesvirus increased the number of chickens with lesions in coronary arteries of all sizes compared with the control groups. Infection with MDV and cholesterol feeding induced coronary lesions in more birds than did virus infection alone.

Microscopic arterial lesions in mesenteric, celiac, and gastric arteries were found only in virus-infected chickens. Nine of 22 Group I chickens and 6 of 9 Group II chickens had arterial lesions compared with none of 13 in Group III and none of 19 in Group IV ($\chi_1^2 = 7.16, 16.12$; $P < 0.01$, $P < 0.001$). The number of chickens with gastric, mesenteric, and celiac arterial lesions was not shown to be significantly different in Group I compared with Group II.

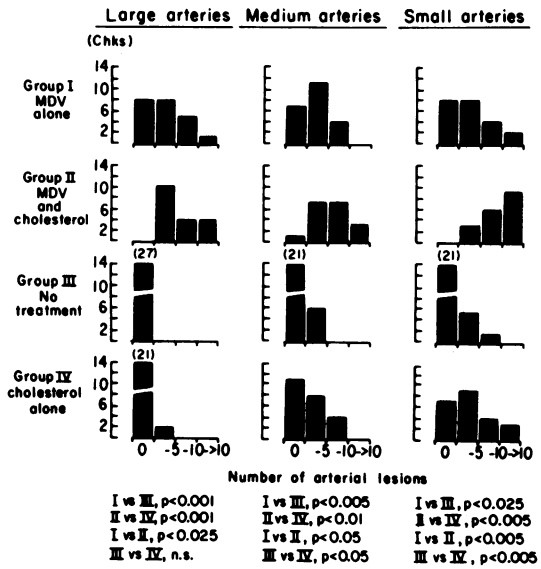
Similarly, microscopic lesions in the proximal portion of the aorta and brachiocephalic arteries were observed almost exclusively in virus-in-

fected chickens (Text-figure 3) ($\chi_1^2 = 25.71, 26.81$; $P < 0.001$). In the mid-portion of the aorta, lesions occurred in uninfected control chickens but at a lower frequency than in virus-infected chickens ($\chi_1^2 = 15.61$; $P < 0.001$). There was a significantly greater number of Group II chickens with arterial lesions in these arterial segments compared with uninfected cholesterol-fed controls, Group IV ($\chi_1^2 = 27.78, 27.78, 21.43$; $P < 0.001$). There were significantly more Group I than Group III chickens with arterial lesions in the proximal descending aorta and brachiocephalic arteries ($\chi_1^2 = 7.47, 7.73$; $P < 0.01$). The number of Group I and Group III chickens with arterial lesions in the mid-portion of the descending aorta was not shown to be significantly different. There were significantly more Group II than Group I chickens with lesions in the brachiocephalic arteries and the proximal and mid-portions of the aorta ($\chi_1^2 = 5.07, 7.68, 11.76$; $P < 0.01$). In contrast, lesions of the distal aorta occurred with similar frequency in all groups. The number of chickens with arterial lesions in the various segments of the aorta was not shown to be significantly different in Groups III and IV.

Coronary Arterial Lesions

Although lesions were found in coronary arteries of chickens in all experimental groups, they were significantly different with respect to the number of chickens with lesions (see above) and their distribution, character, and extent of change.

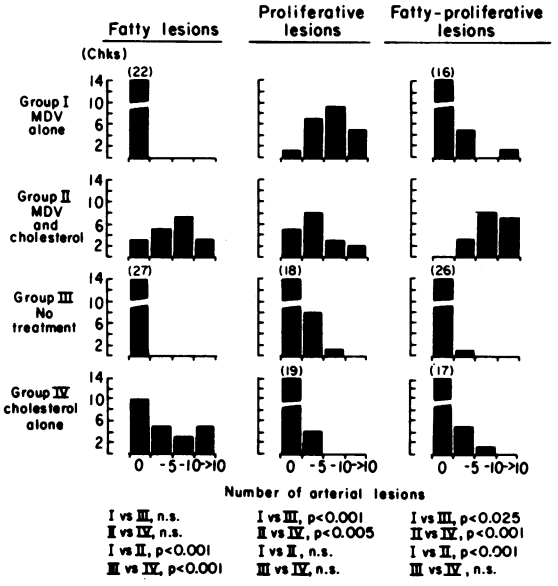
As shown in Text-figure 4, the distribution of arterial lesions among large, medium, and small coronary arteries was different in experimental groups. There were significantly more lesions in large coronary arteries of virus-infected chickens compared with uninfected chickens. Of 22 chickens in Group I, 8 had no lesions in large coronary arteries, 8 had 1 to 4 lesions, 5 had 5 to 10 lesions, and 1 had more than 10 lesions. Of 18 chickens in Group II, 10 had 1 to 4 lesions, 4 had 5 to 10 lesions, and 4 had more than 10 lesions. In contrast, there were no lesions in large coronary arteries in the 27 chickens in Group III and only 2 chickens with one lesion each in Group IV ($\chi_3^2 = 24.1, 34.2$; $P < 0.001$). Among virus-infected chickens there was a significantly greater number of chickens with an increased frequency of lesions in large arteries in Group II compared with Group I ($\chi_3^2 = 9.83$, $P < 0.025$). There were significantly more chickens with an increased frequency of lesions in medium and small coronary arteries in Groups I and II compared with their respective control Groups III and IV (medium arteries: $\chi_3^2 = 12.09, 11.78$; $P < 0.005$, $P < 0.01$) (small arteries: $\chi_3^2 = 9.91, 12.98$; $P < 0.025$, $P < 0.005$). As was the case in large coronary arteries, infection with MDV in choles-



TEXT-FIGURE 4—Distribution of coronary lesions.

terol-fed chickens led to significantly more chickens with an increased frequency of lesions in medium and small arteries compared with infection with virus alone (Group I vs II: $\chi_3^2 = 8.90, 14.88$, $P < 0.05$, $P < 0.005$). Cholesterol feeding alone (Group IV) also increased the number of chickens with an increased frequency of lesions in medium and small coronary arteries compared with chickens in Group III fed a lipid-poor diet ($\chi_3^2 = 7.14, 14.88$; $P < 0.05$, $P < 0.005$). In summary, infection with MDV either with or without cholesterol supplement led to a significantly greater number of arterial lesions in large coronary arteries compared with uninfected chickens fed cholesterol-supplemented diets or cholesterol-poor diets. The number of arterial lesions in medium and small coronary arteries was significantly greater in virus-infected birds of Groups I and II compared with their respective control groups. In coronary arteries of all sizes, virus infection and cholesterol feeding led to significantly more arterial lesions than did virus infection alone.

The microscopic character of coronary arterial lesions was also different in experimental groups (Text-figure 5) (Figures 5 through 10, 23, and 24). Coronary arterial lesions were classified according to type, ie, fatty, proliferative, and fatty-proliferative. The characteristics of these lesions have been previously described.[8] Briefly, fatty arterial lesions were characterized by intracellular and extracellular accumulation of lipid with little cellular proliferation and relatively small quantities of dense collagen (Figure 10). Proliferative lesions were segmental and were characterized



TEXT-FIGURE 5—Character of coronary lesions.

by fibrocellular thickening of the intima and, in some instances, increased numbers of medial cells. The internal elastic lamina often appeared fragmented or, in some instances, reduplicated. Adventitial fibrosis and infiltration of mononuclear cells was occasionally present but was not generally a prominent feature (Figures 5 and 6). Arterial lesions without intimal thickening, which were characterized by focal medial necrosis and increased cellularity of media, were also classified as proliferative lesions (Figure 23). In some instances, proliferative arterial lesions bore close resemblance to human arteriosclerosis without lipid change. Fatty-proliferative lesions were similar to proliferative lesions but had the additional feature of intracellular and extracellular intimal and medial lipid accumulation (Figures 7 through 9). Intima and portions of media in these latter lesions stained red with oil red O stains. Some fatty-proliferative arterial lesions had little or no appreciable intimal thickening, and the predominant change was focal medial necrosis, hypercellularity, and lipid accumulation (Figure 24).

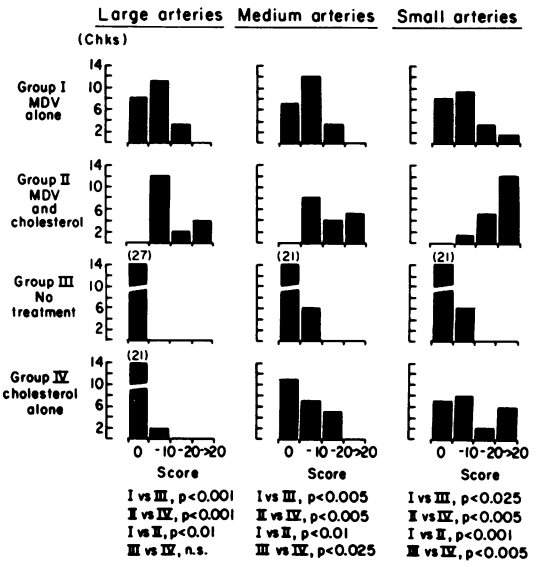
In many instances, fatty-proliferative arterial lesions in coronary arteries and other portions of the arterial system bore striking resemblance to chronic human atherosclerosis. These latter lesions were characterized by collections of foamy fat-containing cells, fatty-hyaline intimal change, and relatively acellular pools of lipid and cholesterol clefts deep in the intima and media with overlying fibromuscular caps (Figures 7, 8, 11–

16, 18, and 19). The intima and subjacent media were often focal calcified, and in some instances the intima was vascularized (Figures 11, 12, and 16). Segmental medial necrosis or scarring with thinning of the media was often observed (Figures 7, 11–13, and 15). Adventitial fibrosis and accumulation of lymphocytes and mononuclear cells were present in some instances but were not often a prominent feature.

Feeding a cholesterol-supplemented diet to uninfected chickens resulted primarily in an increase of fatty arterial lesions (Figure 10). The number of fatty arterial lesions in Group IV was significantly greater than the number in Group III ($\chi_3^2 = 20.62$, $P < 0.001$). It was not possible to demonstrate a significant difference in the number of proliferative and fatty-proliferative arterial lesions in Group III compared with Group IV.

Infection with MDV (Group I) resulted in a significantly greater number of chickens with increased frequency of proliferative and fatty-proliferative coronary lesions (Figures 5 and 6) compared with untreated chickens ($\chi_3^2 = 26.4, 5.5$, $P < 0.001$, $P < 0.025$). There were no fatty coronary arterial lesions in Group I. Feeding cholesterol-supplemented diets to infected chickens of Group II resulted in a significantly greater number of chickens with increased frequency of proliferative and fatty-proliferative lesions compared with cholesterol-fed chickens of Group IV ($\chi_3^2 = 14.10$, 29.78; $P < 0.005$, $P < 0.001$). It was not possible to demonstrate a difference in the frequency of fatty arterial lesions in Group II compared with Group IV or of proliferative arterial lesions in Group II compared with Group I. The number of chickens with increased frequency of fatty-proliferative lesions in Group II was significantly greater than in either Group IV ($\chi_3^2 = 29.78$, $P < 0.001$) or Group I ($\chi_3^2 = 28.89$, $P < 0.001$). Thus, feeding cholesterol-supplemented diet resulted in similar numbers of fatty arterial lesions (Group II vs Group IV), and infection with virus resulted in increased numbers of arterial lesions with proliferative features, either proliferative or fatty-proliferative (Groups I and II).

The extent of coronary arterial change as measured by the degree of occlusion and number of lesions was significantly greater in virus-infected chickens of Groups I and II than in the uninfected chickens of their respective control Groups III and IV (Text-figure 6). In large, medium, and small arteries there were more chickens with greater extent of arterial change in Group I compared with Group III ($\chi_2^2 = 24.05, \chi_2^2 = 11.61, \chi_3^2 = 9.93$; $P < 0.001$, $P < 0.005$, $P < 0.025$). There were more chickens with a greater extent of arterial change in large, medium, and small arteries of Group II compared with Group IV ($\chi_3^2 = 34.03$, 15.63, and 15.35; $P < 0.001$, $P < 0.005$, $P < 0.005$). There were significantly more chickens with



TEXT-FIGURE 6—Extent of coronary arterial lesions.

a greater extent of change in large, medium, and small arteries in Group II compared with Group I (χ_3^2 = 11.96, 12.51, and 24.21; $P < 0.01$, $P < 0.01$, $P < 0.001$). There were also significantly more chickens of Group IV compared with III with greater extent of arterial change in small and medium arteries but not in large arteries (χ^2 = 15.06, 7.93, and 2.45; $P < 0.025$, $P < 0.005$, ns). Thus, when microscopic arterial lesions were considered with respect to size, infection with MDV increased the extent of change in Groups I and II compared with untreated controls (Group III) and cholesterol-fed uninfected chickens. Feeding a cholesterol-supplemented diet to infected chickens of Group II increased the extent of change compared with infected chickens without cholesterol supplement (Group I).

The extent of change was also increased in virus-infected chickens when arterial lesions of similar character were compared. Thus, the number of chickens with a greater extent of arterial change among proliferative lesions was significantly increased in Group I compared with Group III and in Group II compared with Group IV (χ_3^2 = 23.67, 14.10; $P < 0.001$, $P < 0.005$). The number of chickens with a greater extent of arterial change among fatty-proliferative lesions was significantly increased in Group II compared with either Group IV (χ_3^2 = 29.40, $P < 0.001$) or Group I (χ_3^2 = 27.56; $P < 0.001$). It was not possible to demonstrate a significant difference in the extent of arterial change among fatty lesions of Group II compared with Group IV.

Aortic Lesions

Microscopic lesions in the brachiocephalic arteries and in the proximal and mid-portions of the descending aorta were similar with respect to histologic character in all experimental groups. Lesions in these portions of the arterial system were characterized by proliferative or fatty-proliferative intimal thickening often associated with degenerative change or necrosis in the subjacent media (Figures 17–19, 21, 22, 25, and 26). In some instances, medial degeneration appeared to contribute to intimal thickening through a process of medial effacement (Figures 21 and 22). The proportion of aortic lesions that were proliferative and fatty-proliferative of Group I and that of Group II were similar. This finding contrasts with that in coronary arteries, where the greater proportion of lesions were proliferative in Group I compared with II. However, it was our impression that the extent of lipid-accumulation was greater in the fatty-proliferative lesions of Group II compared with Group I (compare Figure 17 with Figures 18 and 19). Lesions in the distal portion of the descending aorta were qualitatively similar in all experimental groups. These latter lesions were different from those in the more proximal aorta. They were characterized by relatively less cellular intimal thickening, with marked thinning of the subjacent media (Figure 20). Occasional foci of extracellular and intracellular lipid were found deep in the intima adjacent to the internal elastic lamina.

As determined by the thickness of the intima, the extent of arteriosclerotic change in the brachiocephalic arteries and proximal and mid-portions of the descending aortas was significantly greater in chickens infected with MDV (Groups I and II) than in the respective control Groups III and IV (Text-figure 7) ($\chi_1^2 = 10.02$, $\chi_3^2 = 21.68$; $P < 0.005$, $P < 0.001$). In the proximal portion of the descending aorta, the number of chickens with increased intimal thickness was also significantly greater in Groups I and II compared with Groups III and IV ($\chi_1^2 = 10.33$, $\chi_4^2 = 21.68$; $P < 0.005$, $P < 0.001$) and in virus-infected cholesterol-fed chickens of Group II compared with chickens of Group I ($\chi_4^2 = 35.5$, $P < 0.001$). In the mid-portion of the descending aorta, the number of chickens with increased intimal thickness could not be shown to be significantly different in Group I chickens compared with untreated controls (Group III). However, there were significantly more chickens with increased intimal thickness in Group II compared with Group IV ($\chi_3^2 = 30.79$, $P < 0.001$) and in Group II compared with Group I ($\chi_3^2 = 12.53$, $P < 0.01$). In the distal portion of the descending aorta, it was not possible to demonstrate a significant difference in the number of chickens with increased intimal thickness when any of the experimental groups were compared.

We were not able to demonstrate significant correlation between the degree of thickness of the intima and media in any of the aortic segments. Thus, in the brachiocephalic arteries and in the proximal and mid-portions of the aorta, the extent of atherosclerosis was significantly greater in virus-infected chickens compared with their respective control groups. Virus infection and cholesterol feeding led to more atherosclerosis than did virus infection alone. We were not able to demonstrate an effect for either virus infection or cholesterol feeding on intimal lesions in the distal aorta.

Other Arterial Lesions

There was appreciable microscopic arterial change in mesenteric, celiac, or gastric arteries of 9 of 20 chickens in Group I and of 6 of 9 chickens in Group II. Microscopic change in Group I chickens consisted of fibromuscular intimal thickening, sometimes occlusive, with lipid deep in the intima and subjacent media (Figures 11 and 12). Similar occlusive change was seen in these arteries in chickens of Group II (Figures 13 through 16). In both instances, atheromata contained extracellular lipid and cholesterol clefts as well as cellular lipid (Figure 15). In some advanced atheromatous lesions, extensive vascularization of the intimal plaques was seen (Figure 16). No microscopic changes were found in these arteries in either the untreated chickens (Group III) or in uninfected chickens fed cholesterol-supplemented diets (Group IV).

Sections of one random block of brachiocephalic artery in each chicken and gastric arteries in some chickens were examined by immunofluorescent microscopy for the presence of Marek's herpesvirus antigen. Specific antigen was observed by immunofluorescence microscopy in the arterial walls of 4 infected birds with gross arterial lesions (Figures 27 and 28). The staining was intracellular and diffuse. The staining was found in long thin cells that were likely to be smooth-muscle cells. Antigen was not identified in arterial tissue of control birds.

Myocardial Lesions

Microscopic lesions were present in the myocardium of chickens of all experimental groups. In uninfected chickens of Groups III and IV, these lesions consisted of poorly defined nodules of intermediate and small lymphocytes. In virus-infected chickens of Groups I and II, there were also patchy infiltrates of small and intermediate-sized lymphocytes (Figure 29). In some chickens there were extensive infiltrations of large lymphocytes. In 2 of 22 chickens of Group I and 3 of 17 chickens of Group II, the infiltrate consisted of immature lymphocytes with large nuclei,

densely clumped chromatin, and prominent nucleoli. These latter infiltrates were associated with myocytolysis and necrosis of adjacent myocardial fibers (Figures 31 and 32). In addition, no gross or microscopic myocardial infarcts were found in any of the groups. There was involvement of myocardium by lymphoid tumors in 2 chickens in Group I.

Myocardial accumulations of lymphocytes were present in a significantly greater number of virus-infected chickens (Groups I and II) than in the respective control Groups III and IV. Myocardial lesions were present in 22 of 28 chickens in Group I and in 17 of 20 chickens in Group II, compared with 10 of 27 chickens in Group III and 8 of 23 chickens in Group IV (χ_1^2 = 9.75, 11.08; $P < 0.005$, $P < 0.001$). The presence or absence of myocardial infiltration and the degree of change was scored as outlined in Materials and Methods. The number of chickens with increased myocardial infiltration was significantly greater in virus-infected chickens (Groups I and II) than in the respective control Groups III and IV (χ_4^2 = 11.64, 16.65; $P < 0.025$, $P < 0.005$). It was not possible to demonstrate a significant difference in the extent of myocardial change either between Group I and Group II or between Group III and Group IV.

Nerve Lesions

Infiltration of nerves by lymphocytes was seen in 24 of 28 chickens in Group I and 16 of 20 chickens in Group II (Figure 30). Microscopically, lesions of nerves were never seen in Groups III and IV (Groups I and II vs III and IV: χ_4^2 = 41.06, 29.03; $P < 0.001$). In Group II there was a significant positive correlation between the number of coronary lesions and the score of myocardial or nerve lesions (r = 0.63, 0.57; $P < 0.01$, $P < 0.02$, respectively). In Group I there was a weak nonsignificant correlation between the number of coronary lesions and the number of myocardial or nerve lesions (r = 0.24, 0.14; ns).

Discussion

Infection with Marek's herpesvirus leads to atherosclerosis. The following observations support this conclusion: 1) Infection with MDV induced arterial lesions in portions of the arterial tree that were not involved in either untreated control chickens or in chickens fed cholesterol-supplemented diets, ie, large coronary arteries, proximal aortas, brachiocephalic arteries, and celiac, gastric, and mesenteric arteries. 2) Infection with MDV led to an increased incidence of arterial lesions in portions of the arterial tree that were involved in chickens of Groups III and IV, ie, medium and small coronary arteries. 3) Infection with MDV induced

arterial lesions of different character than those induced by cholesterol feeding. 4) The extent of atherosclerosis was greater in virus-infected chickens of either groups than in the respective control groups. 5) It was not possible to show a correlation between serum cholesterol concentration and the number of coronary lesions in Group II, although a strong and significant positive correlation was present in chickens of Group IV.

It has been suggested that virus infections may be important in the pathogenesis of atherosclerosis in humans.[35] There is extensive evidence to indicate that viruses may lead to arterial disease in humans and animals. Infection with hepatitis B virus and Coxsackie viruses have been implicated in arterial disease in humans.[36,37] Infection with viruses has also been shown to lead to arterial damage in immune complex disease in New Zealand mice,[38] lymphocytic choriomeningitis virus infection in mice,[39] Coxsackie virus and encephalomyocarditis virus infections in mice,[40,41] Pichinde virus infections of hamsters,[42] Aleutian disease of mink,[43] equine viral arteritis and infectious anemia,[44,45] African swine fever and hog cholera,[46] bovine malignant catarrhal fever and mucosal disease of cattle,[47,48] Border disease of sheep,[49] and Bolivian hemorrhagic fever in nonhuman primates.[50] Results of our experiments indicate that infection with a herpesvirus can lead to atherosclerosis in chickens that closely resembles that in humans.[23,24] In this regard it is important to note that there is widespread infection of human populations with up to five herpesviruses. These viruses are herpes simplex 1 and 2,[51,52] varicella zoster,[53] Epstein–Barr virus,[54] and the cytomegaloviruses.[55] Infections with herpesviruses are well known to persist for long periods as latent or reactivated latent infections.[56,57]

Results of experiments by Paterson et al suggest that arterial disease resulting from an infectious agent, presumably a virus, may be important in the pathogenesis of chicken atherosclerosis.[29,58,59] Paterson et al observed 1) that lipid accumulation in chicken coronary arteries appeared to occur at sites of previously induced arterial damage, 2) that the chickens used had neurolymphomatosis, and 3) that the incidence of coronary lesions could be increased by inoculating chickens with a relatively low incidence of neurolymphomatosis with tracheal washings from chickens with a high incidence. Paterson and co-workers concluded that an infectious agent associated with neurolymphomatosis led to arterial damage which predisposed to lipid deposition in the arterial wall. At this time it was not possible to prove an association, because the etiologic agent of neurolymphomatosis, ie, Marek's disease herpesvirus, had not been isolated and because chickens free of neurolymphomatosis were not available. Results of experiments reported here indicate that infection with the

etiologic agent of neurolymphomatosis, ie, MDV, will lead to atherosclerosis in chickens that are free of all known viral and microbial pathogens. We know of no other experiments in which atherosclerosis has been shown to be induced by infection with a virus.

The pathogenesis of the proliferative and fatty-proliferative arterial lesions in virus-infected chickens of Groups I and II has not been elucidated. Conceivably, these arterial lesions could result from one or more of the following: 1) arterial injury or a reaction to the injury, 2) virus-stimulated proliferation of vascular smooth muscle cells, 3) oncogenic transformation of smooth muscle cells, or 4) the effect of virus on lipid metabolism. Since virus infection without dietary cholesterol supplement induces predominantly proliferative arterial lesions (see Results and Text-figure 5), it appears unlikely that alterations of lipid metabolism are the only pathogenic factor. Changes in lipid metabolism may contribute to the character and, perhaps, the progression of some lesions.

The presence of Marek's disease specific viral antigen in arterial walls of chickens with arterial lesions suggests that MDV is directly involved in the pathogenesis of the lesions. Findings with immunofluorescent microscopy also suggest that the virus is contained in vascular smooth-muscle cells. Diffuse cytoplasmic staining of Marek's disease specific antigen within smooth muscle cells in the arterial wall could indicate that the arterial cells either contain virus particles or are producing viral antigens. According to the Roizman doctrine, such productively infected cells would be expected to die.[60] This virally mediated cell injury might be the origin of the areas of medial necrosis described in the next paragraph. Conversely, herpesvirus-transformed cells would not be expected to produce viral internal antigen that would be detected by fluorescent antibody.[61]

In some chickens of Groups I and II, aortas, coronary arteries, and mesenteric arteries without intimal thickening were found to have exquisitely focal areas of medial necrosis and lymphocytic infiltrate often associated with lipid-laden foam cells. Arterial smooth-muscle cells immediately adjacent to these areas of change were often ostensibly normal. The overlying media and endothelium appeared to be intact by light microscopy. Medial necrosis and reactive change in arteries without intimal thickening suggest that medial injury may be important in the pathogenesis of these arterial lesions and that foci of medial necrosis may precede intimal thickening. The presence of similar medial lesions in arteries of chickens that died a few weeks after infection in other experiments supports this possibility.[62] Although morphologic findings obtained by light microscopy suggest that medial injury is the earliest change in

coronary arteries and aortas, it is not possible to exclude a role for endothelial injury and repair by techniques used in these experiments.

It is reasonable to consider the possibility that endothelial injury may be important in the pathogenesis of these virus-induced lesions since at least one group of herpesvirus, ie, cytomegaloviruses, have been shown to involve endothelium in humans.[63-65] Viruses have been described in endothelial cells in human Coxsackie myocarditis.[66] Burch et al demonstrated endothelial degeneration in association with Coxsackie B_4 and encephalomyocarditis virus infections in mice.[40,41] Several other viruses have been found to infect endothelium in experimental animals.[67-69] Endothelial injury and repair have been implicated in several experimental models of atherosclerosis that result from mechanical,[4,7,15] chemical,[5,10,13] or immunologic injury.[15,70] Although we have no evidence to indicate that loss of endothelium is important in the pathogenesis of these lesions, careful examination of endothelium by scanning and transmission electron microscopy over the time span of lesion development will be necessary to rule out the possibility of endothelial injury.

Arterial injury could result from a direct cytopathic effect of the herpesvirus on the arterial smooth-muscle cells and/or endothelial cells, as indicated above. Alternatively, arterial injury may be mediated indirectly. In this regard, a possible role for immunologic arterial injury should be considered. Immunologic responses are believed to be important in the pathogenesis of Marek's disease.[61] Immunologic injury to the arterial wall has been implicated in atherosclerosis in humans.[35,36,71-78] Humans are exposed to many foreign substances that are antigenic; eg, it has been suggested that immunologic mechanisms may be important in understanding the role of tobacco as a risk factor.[77] Studies by Harkavy and Perlman [78] indicated that patients with coronary disease who smoked have an increased incidence of positive skin tests to tobacco leaf protein, as compared with smokers without coronary artery disease. Becker et al [77] isolated a small glycoprotein from cured tobacco leaves and from cigarette smoke condensate to which approximately one third of smokers and nonsmokers exhibit immediate cutaneous hypersensitivity. Immunologically mediated arterial injury due to either immune complex disease or graft rejection has induced atherosclerosis, often closely resembling that in humans, in several species of experimental animals.[8,9,14,79,80] Finally, in humans [36] and animals, immunologic response to viruses has been shown to be important to the genesis of arterial disease.[38,39,43,44] Immunologic arterial injury related to MDV infection could result from immune complex disease or from cell-mediated immune injury. The focal nature of

both the viral antigen and the arterial necrosis as well as the association of the latter with lymphocytes are suggestive of cell-mediated immune injury. Myocardial necrosis in virus-infected chickens was also found to be associated with lymphocytes and may be analogous.

On the other hand, the intimal thickening in the arterial lesions could result from proliferation of medial smooth muscle cells stimulated by virus infection. Herpes simplex viruses have been found to lead to marked proliferation and piling of infected cells in culture.[81] Marek's disease virus infection of duck embryo fibroblasts leads to cell proliferation.[82] We have observed that MDV will lead to proliferation of vascular smooth muscle cells in culture.[83] Alternatively, the proliferation may result from oncogenic transformation of cells, since MDV is an oncogenic virus. This latter mechanism for cellular proliferation may support the hypothesis of Benditt that atherosclerotic lesions are analogous to neoplasms.[20] The exquisitely focal nature of some grossly visible arterial plaques and proliferative and fatty-proliferative microscopic arterial lesions is in keeping with either of these interpretations. The ability of Marek's disease herpesvirus to induce cell proliferation and the oncogenicity of Marek's disease viruses in chickens continue to make these possibilities attractive.

There was a significant positive correlation between nerve lesions, myocardial lesions, and the incidence of coronary arterial lesions in virus-infected, cholesterol-fed chickens of Group II but not of Group I. This discrepancy in the two virus-infected groups may result from the increased incidence of arterial lesions in Group II compared with Group I. In the instance of nerve lesions, such a correlation could either indicate an association between one late manifestation of Marek's disease, ie, nerve lesions and vascular lesions, or indicate that involvement of nerves may have a direct effect on the arterial wall and a primary role in the arterial lesions. The possibility exists that denervation of arteries is associated with metabolic change in the arterial wall and increased atherogenesis.[84,85] The correlation with myocardial lesions is of interest since in both instances there is focal necrosis of muscle associated with lymphocytes. In contrast, arterial lesions in chickens of Group II could not be shown to correlate with the concentration of serum cholesterol. Thus, arterial lesions in Group II correlate with two manifestations of Marek's disease, ie, myocardial and nerve lesions, but not with serum cholesterol concentrations.

The number of chickens with arterial lesions as well as the number of coronary arterial lesions and the extent of coronary and aortic lesions were significantly greater in chickens of Group II compared with chickens of Group I. Taken together, these findings suggest that infection with MDV and cholesterol feeding act synergistically to lead to an increased in-

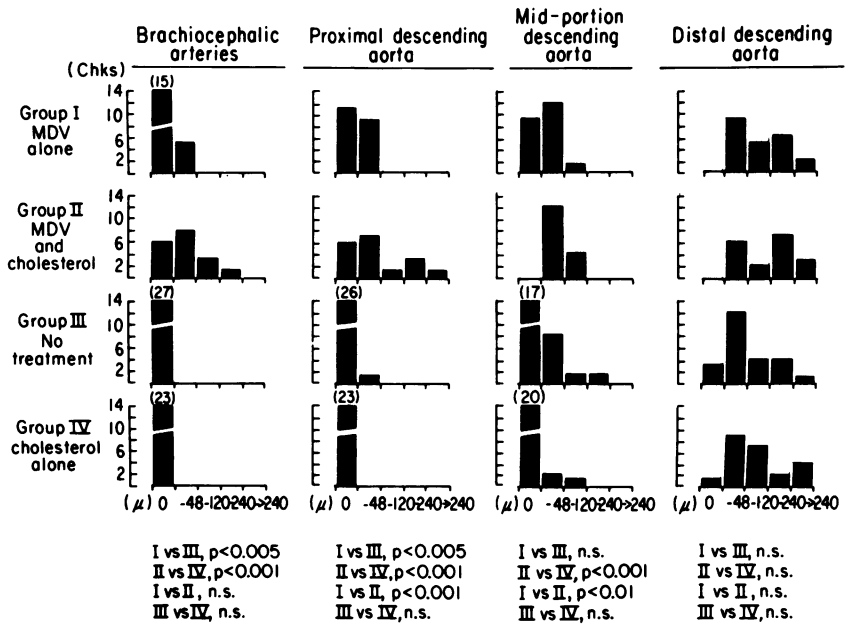
cidence of both lesions and extent of arterial change. The mechanism of the synergistic effect is not clear. It is conceivable that many viral-induced arterial lesions undergo repair and heal spontaneously in the presence of normal serum cholesterol concentrations. In this regard, Ross and Glomset[12] found that in nonhuman primates intimal thickening, resulting from balloon catheter injury, regressed with time, although results of recent experiments of Spaet et al [86] in rabbits are at variance with this finding. In virus-infected chickens fed cholesterol supplements, increased lipid or lipoprotein in the viral-induced lesions may either inhibit these normal reparative processes or stimulate cell proliferation and in this manner enhance progression of arterial lesions. This is consistent with the finding of others that low-density lipoproteins (in particular, low-density lipoproteins from hypercholesterolemic serum) stimulate smooth muscle proliferation *in vitro*.[87,88] Alternatively, hypercholesterolemia and accompanying metabolic effects may alter the response of the arterial tissue to the virus and enhance the effect of virus on the arterial wall. Experiments of Campbell et al [89] demonstrated that the synergy of Cosackie viral infections and hypercholesterolemia in mice led to myocytolysis in myocardium and degenerative lesions in the aorta that were not found in virus-infected normocholesterolemic mice. Findings of these experiments in chickens suggest that persistent herpesvirus infections alone may induce arteriosclerosis in humans and that known risk factors such as hypercholesterolemia may act synergistically to increase the extent of the atherosclerosis.

Chronic atheromatous lesions with significant quantities of lipid were present in several portions of the arterial system in normocholesterolemic, virus-infected chickens of Group I. Although the lipid accumulation was usually greater in chickens fed cholesterol supplement, this degree of atheromatous change in virus-infected normocholesterolemic Group I chickens was unexpected and suggested that infection with the virus had an effect on lipid metabolism within the arterial wall. Among many possibilities, viruses may alter arterial lipid metabolism by one or more of the following: 1) increasing serum lipid as a result of altered lipoprotein metabolism and lipid clearance, 2) by alteration of metabolism of lipids by cells within the arterial wall, 3) by injuring endothelium and increasing lipid transport into the arterial wall, and 4) by altering the structure of the arterial intima and thereby enhancing lipid accumulation. We were not able to demonstrate a significant difference in serum cholesterol concentration in cholesterol-fed animals of Group IV and virus-infected, cholesterol-fed animals of Group II. The exquisitely focal nature of what we interpret to be early arterial lesions suggests that local effects induced in

the arterial wall by the effect of the virus on vascular smooth-muscle cells may be more important in lipid accumulation in the arterial lesions of Group I and II animals than effects of the infection on overall lipid metabolism of the host. Herpesvirus infection may alter lipid metabolism of host cells. Fabricant et al [21] demonstrated accumulation of lipid globules and crystalline material in Crandell feline kidney cells infected with a feline herpesvirus. Similar lipid globules and crystalline material were seen in culture fluids. Lipid globules in cells stained with oil red O indicate neutral lipid. Mass spectrums of crystals from cultures were found to correspond to those in a cholesterol standard.[21] Results of experiments of Mark-Malchoff et al [22] indicate that infection with polyoma virus may lead to changes in cholesterol esterification and a decrease in cholesterol ester hydrolysis. Preliminary evidence from our experiments indicates that chicken vascular smooth-muscle cells in culture infected with MDV have altered lipid metabolism.[90]

Experiments of Friedman and Moore indicate that sustained or repeated endothelial injury may lead to lipid accumulation in normocholesterolemic rabbits.[91,92] Since chickens can be shown to be persistently infected with Marek's virus, the possibility exists that sustained viral injury to the endothelium may contribute to the intimal lipid accumulation in these experiments. Finally, viruses have been shown to modify the synthesis of glycosaminoglycans by cultured cells. Glycosaminoglycans have been shown to bind to low-density lipoproteins both *in vitro* and *in vivo* and may be important in atherogenesis in animals.[93-96] Thus, infection with Marek's virus could increase the synthesis of glycosaminoglycans or qualitatively alter the glycosaminoglycans and enhance intimal lipid accumulation.

In most portions of the arterial system, infection with MDV had a significant effect on the incidence and extent of arterial lesions. However, in the distal aorta the number and severity of lesions in virus-infected chickens was not significantly increased compared with uninfected control chickens. Since there were appreciable lesions in distal aortas of untreated chickens, these are apparently different from virus-induced lesions and represent spontaneously occurring lesions due to unknown factors. Similar lesions have been described by others in the distal aorta of chickens and pigeons.[97-99] Results of experiments of Albert et al indicate that injections of two polycyclic hydrocarbons, ie, 7,12-dimethylbenz(a,h)anthracene and benzo(a)pyrene, increased the incidence and severity of arterial lesions of the abdominal aorta in chickens. These investigators suggested that the polycyclic hydrocarbons produced effects on the atherosclerotic process in chickens analogous to the effect of these substances in promot-



TEXT-FIGURE 7—Thickness of aortic intimal lesions.

ing shortened appearance time for spontaneously occurring smooth-muscle tumors in animals.[100] As illustrated in Text-figures 3 and 7, we were unable to demonstrate an effect of infection with Marek's herpesvirus on either the incidence or severity of the lesions in the distal aorta of chickens.

Infection with Marek's herpesvirus induced arterial lesions that bore close resemblance to chronic atherosclerosis in humans, both in chickens fed cholesterol-supplemented and in chickens fed cholesterol-poor diets. It is remarkable that lesions which so closely resemble chronic human atherosclerosis, a disease which we conceive of as taking years to evolve, were induced in hypercholesterolemic as well as normocholesterolemic chickens in a few months. Although the striking morphologic similarity between the arterial lesions in these chickens and atheroarteriosclerosis in humans may be fortuitous, the importance of viruses in arterial disease in humans and other animals, together with results of these experiments, strongly supports the possibility that persistent viral infections acting alone or in synergy with other known risk factors may lead to atherosclerosis in humans. Results of these experiments may be especially pertinent to our understanding of the etiology and pathogenesis of human atherosclerosis, since there is widespread and persistent infection of human

populations with up to five herpesviruses. The rapid evolution of chronic atherosclerosis in these chickens provides unusual opportunities for investigating the pathobiologic characteristics of human atherosclerosis.

References

1. Gordon T, Garcia-Palmieri MR, Kagan A, Kannel WB, Schiffman J: Differences in coronary heart disease in Framingham, Honolulu and Puerto Rico. J Chronic Dis 27:329–344, 1974
2. Anitschkow NN: Experimental arteriosclerosis in animals. Arteriosclerosis: A Survey of the Problem. Edited by EV Cowdry. New York, Macmillan Publishing Co, 1933, pp 271–322
3. Duff GL: Experimental cholesterol arteriosclerosis and its relationship to human arteriosclerosis. Arch Pathol 20:80–123; 259–304, 1935
4. Taylor CB: The reaction of arteries to injury by physical agents. With a discussion of arterial repair and its relationship to atherosclerosis. Symposium on Atherosclerosis. Washington, DC, National Academy of Sciences, 1955, pp 74–90
5. Waters LL: The reaction of the artery wall to injury by chemicals or infection,[4] pp 91–98
6. Minick CR, Murphy GE, Campbell WG Jr: Experimental induction of atheroarteriosclerosis by the synergy of allergic injury to arteries and lipid-rich diet: I. Effect of repeated injections of horse serum in rabbits fed a dietary cholesterol supplement. J Exp Med 124:635–652, 1966
7. Stemerman MB, Ross R: Experimental arteriosclerosis: I. Fibrous plaque formation in primates: An electron microscopic study. J Exp Med 136:769–789, 1972
8. Minick CR, Murphy GE: Experimental induction of atheroarteriosclerosis by the synergy of allergic injury to arteries and lipid-rich diet: II. Effect of repeatedly injected foreign protein in rabbits fed a lipid-rich, cholesterol-poor diet. Am J Pathol 73:265–300, 1973
9. Hardin NJ, Minick CR, Murphy GE: Experimental induction of atheroarteriosclerosis by the synergy of allergic injury to arteries and lipid-rich diet: III. The role of earlier acquired fibromuscular intimal thickening in the pathogenesis of later developing atherosclerosis. Am J Pathol 73:301–326, 1973
10. Harker LA, Slichter SJ, Scott CR, Ross R: Homocystinemia: Vascular injury and arterial thrombosis. N Engl J Med 291:537–543, 1974
11. Minick CR: Immunologic arterial injury in atherogenesis. Ann NY Acad Sci 275:210–227, 1976
12. Ross R, Glomset JA: The pathogenesis of atherosclerosis. N Engl J Med 295:369–377; 420–425, 1976
13. Ross R, Harker LA: Hyperlipidemia and atherosclerosis: Chronic hyperlipidemia initiates and maintains lesions by endothelial cell desquamation and lipid accumulation. Science 193:1094–1100, 1976
14. Alonso DR, Starek PK, Minick CR: Studies on the pathogenesis of atheroarteriosclerosis induced in rabbit cardiac allografts by the synergy of graft rejection and hypercholesterolemia. Am J Pathol 87:415–442, 1977
15. Minick CR, Stemerman MB, Insull W Jr: Effect of regenerated endothelium on lipid accumulation in the arterial wall. Proc Natl Acad Sci USA 74:1724–1728, 1977
16. Minick CR, Stemerman MB, Insull W Jr: Role of endothelium and hypercholesterolemia in intimal thickening and lipid accumulation. Am J Pathol 95:131–158, 1979
17. Benditt EP, Benditt JM: Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 70:1753–1756, 1973

18. Pearson TA, Kramer EC, Solez K, Heptinstall RH: The human atherosclerotic plaque. Am J Pathol 86:657–664, 1977
19. Pearson TA, Solez K, Dillman J, Heptinstall RH: Monoclonal characteristics of organizing arterial thrombi: Significance in the origin and growth of human atherosclerotic plaques. Lancet 1:7–11, 1979
20. Benditt EP: Implications of the monoclonal character of human atherosclerotic plaques. Ann NY Acad Sci 275:96–100, 1976
21. Fabricant CG, Krook L, Gillespie JH: Virus-induced cholesterol crystals. Science 181:566–567, 1973
22. Mark-Malchoff D, Marinetti GV, Hare JD, Meisler A: Cholesterol content and metabolism in normal and polyoma virus-transformed hamster embryo fibroblasts. Exp Cell Res 118:377–381, 1979
23. Fabricant CG, Fabricant J, Litrenta MM, Minick CR: Virus-induced atherosclerosis in chickens (abstr). Circulation 56(Suppl 3):144, 1977
24. Fabricant CG, Fabricant J, Litrenta MM, Minick CR: Virus-induced atherosclerosis. J Exp Med 148:335–340, 1978
25. Cole RK: Studies on genetic resistance to Marek's disease. Avian Dis 12:9–28, 1968
26. Smith MW, Calnek BW: Comparative features of low-virulence and high-virulence Marek's disease virus infections. Avian Pathol 3:229–246, 1974
27. Kim E, Goldberg M: Serum cholesterol assay using a stable Liebermann-Burchard reagent. Clin Chem 15:1171–1179, 1969
28. Fabricant J, Ianconescu M, Calnek BW: Comparative effects of host and viral factors on early pathogenesis of Marek's disease. Infect Immun 16:136–144, 1977
29. Paterson JC, Slinger SJ, Gartley KM: Experimental coronary sclerosis: I. Medial degeneration as the primary lesion in coronary sclerosis of cockerels. Arch Pathol 45:306–318, 1948
30. Burkholder PM, Littell AH, Klein PG: Sectioning at room temperature of unfixed tissues, frozen in a gelatin matrix, for immunohistologic procedures. Stain Technol 36:89–91, 1961
31. Calnek BW: Effects of passive antibody on early pathogenesis of Marek's disease. Infect Immun 6:193–198, 1972
32. Horlick L, Katz LN: The relationship of atheromatosis development in the chicken to the amount of cholesterol added to the diet. Am Heart J 38:336–349, 1949
33. Calnek BW: Antibody development in chickens exposed to Marek's disease virus. Oncogenesis and Herpesviruses. Edited by PM Biggs, G de-Thé, LN Payne. New York, Elsevier, 1972, pp 129–136
34. Calnek BW: Personal communication, 1977
35. Burch GE: Viruses and arteriosclerosis. Am Heart J 87:407–412, 1974
36. Michalak T: Immune complexes of hepatitis B surface antigen in the pathogenesis of periarteritis nodosa. Am J Pathol 90:619–632, 1978
37. Burch GE, Harb JM, Hiramoto Y, Shewey L: Viral infection of the aorta of man associated with early atherosclerotic changes. Am Heart J 86:523–534, 1973
38. Yoshiki T, Mellors RC, Strand M, August JT: The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune complex glomerulonephritis of New Zealand mice. J Exp Med 140:1011–1027, 1974
39. Oldstone MBA, Dixon FJ: Immune complex disease in chronic viral infections. Proceedings of a Symposium on Immune Complexes and Disease. J Exp Med 134:32S–40S, 1971
40. Burch GE, Tsui CY, Harb JM: Pathologic changes of aorta and coronary arteries of mice infected with Coxsackie B_4 virus. Proc Soc Exp Biol Med 137:657–661, 1971

41. Burch GE, Rayburn P: EMC viral infection of the coronary blood vessels in newborn mice: Viral vasculitis. Br J Exp Pathol 58:565–571, 1977
42. Murphy FA, Buchmeier MJ, Rawls WE: The reticuloendothelium as the target in a virus infection: Pichinde virus pathogenesis in two strains of hamsters. Lab Invest 37:502–515, 1977
43. Porter DD, Larsen AE, Porter HG: The pathogenesis of Aleutian disease of mink: III. Immune complex arteritis. Am J Pathol 71:331–344, 1973
44. Crawford PB, Henson JB: Immunofluorescent, light-microscopic and immunological studies of equine viral arteritis. Proceedings of the Third International Conference of Equine Infectious Diseases. Edited by JT Bryans, H Gerber. Basel, S Karger, 1972, pp 282–302
45. Konno S, Yamamoto H: Pathology of equine infectious anemia: Proposed classification of pathologic types of disease. Cornell Vet 60:393–449, 1970
46. Maurer FD, Griesemer RA: The pathology of African swine fever: A comparison with hog cholera. Am J Vet Res 19:517–539, 1958
47. Liggitt HD, DeMartini JC, McChesney AE, Pierson RE, Storz J: Experimental transmission of malignant catarrhal fever in cattle: Gross and histopathologic changes. Am J Vet Res 39:1249–1257, 1978
48. Ramsey FK: Pathology of a mucosal disease of cattle. Doctoral thesis, Veterinary Pathology Department and Iowa Veterinary Medical Research Institute, Iowa State College, Ames, Iowa, 1956
49. Zakarian B, Barlow RM, Rinnie JC, Head KW: Periarteritis in experimental Border disease of sheep: II. Morphology and histochemistry of the lesion. J Comp Pathol 86:477–487, 1976
50. McLeod CG Jr, Stookey JL, Eddy GA, Scott SK: Pathology of chronic Bolivian hemorrhagic fever in the rhesus monkey. Am J Pathol 84:211–224, 1976
51. Nahmias AJ, Josey WE, Naib ZM, Freeman MG, Fernandez RJ, Wheeler JH: Perinatal risk associated with maternal genital herpes simplex virus infection. Am J Obstet Gynecol 110:825–837, 1971
52. Rawls WE: Herpes simplex virus. The Herpesviruses. Edited by AS Kaplan. New York, Academic Press, 1973, pp 291–319
53. Gold E, Nankervis GA: Varicella-zoster viruses,[52] pp 327–351
54. Klein G: The Epstein-Barr virus.[52] pp 521–555
55. Weller TH, Hanshaw JB: Virologic and clinical observations on cytomegalic inclusion disease. N Engl J Med 266:1233–1244, 1962
56. Rapp F, Jerkafsky MA: Persistent and latent infections,[52] pp 271–289
57. Stevens JC, Cook ML: Latent herpes simplex in sensory ganglia. Perspect Virol 8:171–184, 1973
58. Paterson JC, Mitchell CA. Wallace AC: Experimental coronary sclerosis: II. The role of infection in coronary sclerosis of cockerels. Arch Pathol 47:335–339, 1949
59. Paterson JC, Cottral GE: Experimental coronary sclerosis: III. Lymphomatosis as a cause of coronary sclerosis in chickens. Arch Pathol 49:699–709, 1950
60. Roizman B: The biochemical features of herpesvirus-infected cells, particularly as they relate to their potential oncogenicity: A review. Oncogenesis and Herpesviruses. Edited by PM Biggs, G de-Thé, LN Payne. New York, Elsevier Publishing Co, 1972, pp 1–17
61. Payne LN, Frazier JA, Powell PC: Pathogenesis of Marek's disease. Int Rev Exp Pathol 16:59–154, 1976
62. Minick CR, Fabricant CG, Fabricant J, Litrenta MM: Unpublished observations, 1979
63. Haymaker W, Girdany BR, Stephens J, Lillie RD, Fetterman GH: Cerebral involvement with advanced periventricular calcification in generalized cytomegalic inclusion disease in the newborn. J Neuropath Exp Neurol 13:562–586, 1954

64. Smith MG: The salivary gland viruses of man and animals (cytomegalic inclusion disease). Prog Med Virol 2:171–202, 1959
65. Ward PH, Lindsay JR, Warner NE: Cytomegalic inclusion disease affecting the temporal bone. Laryngoscope 75:628–638, 1965
66. Hass JE, Yunis EJ: Viral crystalline arrays in human Coxsackie myocarditis. Lab Invest 23:442–446, 1970
67. Margolis G, Jacobs LR, Kilham L: Oxygen tension and the selective tropism of K-virus for mouse pulmonary endothelium. Am Rev Resp Dis 114:45–51, 1976
68. Kilham L, Margolis G: Spontaneous hepatitis and cerebellar "hypoplasia" in suckling rats due to congenital infections with rat virus. Am J Pathol 49:457–475, 1966
69. Margolis G, Kilham L: Parvovirus infections, vascular endothelium, and hemorrhagic encephalopathy. Lab Invest 22:478–488, 1970
70. Sharma HM, Geer JC: Experimental aortic lesions of acute serum sickness in rabbits. Am J Pathol 88:255–266, 1977
71. Saphir O, Gore I: Evidence for an inflammatory basis of coronary arteriosclerosis in the young. Arch Pathol 49:418–426, 1950
72. Zeek P: Studies in atherosclerosis: I. Conditions in childhood which predispose to the early development of arteriosclerosis. Am J Med Sci 184:350–356, 1932
73. Zak FG, Helpern M, Adlersberg D: Stenosing coronary arteritis: Its possible role in coronary artery disease. Angiology 3:289–305, 1952
74. Murphy GE: Observations indicating that rheumatic injury to coronary arteries leads in some cases to precocious or later developing atheroarteriosclerosis of these arteries: A microscopic study of coronary arteries of infants, children, adolescents and adults with rheumatic heart disease. Unpublished data, 1973
75. Bulkley BH, Roberts WC: The heart in systemic lupus erythematosus and the changes induced in it by corticosteriod therapy: A study of 36 necropsy patients. Am J Med 58:243–264, 1975
76. Rider AK, Copeland JG, Hunt SA, Mason J, Specter MJ, Winkle RA, Bieber CP, Billingham ME, Dong E Jr, Griepp RB, Schroeder JS, Stinson EB, Harrison DC, Shumway NE: The status of cardiac transplantation, 1975. Circulation 52:531–539, 1975
77. Becker CG, Dubin T, Wiedeman HP: Hypersensitivity to tobacco antigen. Proc Natl Acad Sci USA 73:1712–1716, 1976
78. Harkavy J, Perlman E: Tobacco allergy in coronary artery disease. NY State J Med 64:1287–1296, 1964
79. Howard AN, Patelski J, Bowyer DE, Gresham GA: Atherosclerosis induced in hypercholesterolemic baboons by immunological injury; and the effects of intravenous polyunsaturated phosphatidyl choline. Atherosclerosis 14:17–29, 1971
80. Alexander NJ, Clarkson TB: Vasectomy increases the severity of diet-induced atherosclerosis in *Macaca fasicularis*. Science 201:538–541, 1978
81. Hinze HC, Walker DL: Occurrence of focal three-dimensional proliferation in cultured human cells after prolonged infection with herpes simplex virus. J Exp Med 113:885–898, 1961
82. Spencer JL: Marek's disease herpesvirus: Comparison of foci (macro) in infected duck embryo fibroblasts under agar medium with foci (micro) in chicken cells. Avian Dis 14:565–578, 1970
83. Fabricant CG: Unpublished observations, 1979
84. Murphy TO, Haglin JJ, Felder DA: The progression of experimental atherosclerosis after lumbar sympathectomy. Surg Forum 7:332–336, 1956
85. Anliker M, Anschutz F, Brodie B, Erdos E, Friedman M, Goth A, Gregg D, Gunn CG, Hauss WH, Schneider RA: Endocrine, chemical and neural regulators and the effects of aging in the artery and the process of arteriosclerosis. Adv Exp Med Biol 16A:53–80, 1970

86. Spaet TH, Rhee C, Geiger C: Delayed consequences of endothelial removal from rabbit aortae. Adv Exp Med Biol 102:165–173, 1978
87. Ross R, Glomset JA: Atherosclerosis and the arterial smooth muscle cell. Science 180:1332–1339, 1973
88. Fisher-Dzoga K, Chen R, Wissler RW: Effects of serum lipoproteins on the morphology, growth, and metabolism of arterial smooth muscle cells. Adv Exp Med Biol 43:299–311, 1974
89. Campbell AE, Loria RM, Madge GE: Coxsackievirus B cardiopathy and angiopathy in the hypercholesterolemic host. Atherosclerosis 31:295–306, 1978
90. Fabricant CG, Fabricant J, Hajjar D, Minick CR: Unpublished observations, 1979
91. Friedman RJ, Moore S, Singal DP: Repeated endothelial injury and induction of atherosclerosis in normolipemic rabbits by human serum. Lab Invest 32:404–415, 1975
92. Moore S, Ihnatowycz IO: Vessel injury and atherosclerosis. Adv Exp Med Biol 102:145–164, 1978
93. Satoh C, Duff R, Rapp F, Davidson EA: Production of mucopolysaccharides by normal and transformed cells. Proc Natl Acad Sci USA 70:54–56, 1973
94. Iverius PH: The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. J Biol Chem 247:2067–2613, 1972
95. Woodard JF, Srinivasan SR, Zimny ML, Radhakrishnamurthy B, Berenson GS: Electron microscopic features of lipoprotein-glycosaminoglycan complexes from human atherosclerotic plaques. Lab Invest 34:516–521, 1976
96. Curwen KD, Smith SC: Aortic glycosaminoglycans in atherosclerosis-susceptible and -resistant pigeons. Exp Mol Pathol 27:121–133, 1977
97. Siller WG: Spontaneous atherosclerosis in the fowl. Comparative atherosclerosis. The morphology of spontaneous and induced atherosclerotic lesions in animals. Edited by JC Roberts Jr, R Straus. New York, Hoeber Medical Division, Harper and Row, Publishers, Inc, 1965, pp 66–76
98. Moss NS, Benditt EP: The ultrastructure of spontaneous and experimentally induced arterial lesions: II. The spontaneous plaque in the chicken. Lab Invest 23:231–245, 1970
99. Santerre RF, Wight TN, Smith SC, Brannigan D: Spontaneous atherosclerosis in pigeons. Am J Pathol 67:1–22, 1972
100. Albert RE, Vanderlaan M, Burns FJ, Nishizumi M: Effect of carcinogens on chicken atherosclerosis. Cancer Res 37:2232–2235, 1977
101. Minck CR, Fabricant CG, Fabricant J, Litrenta MM: Unpublished observations

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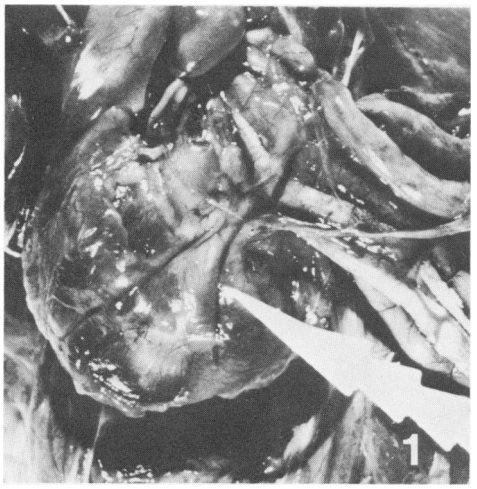
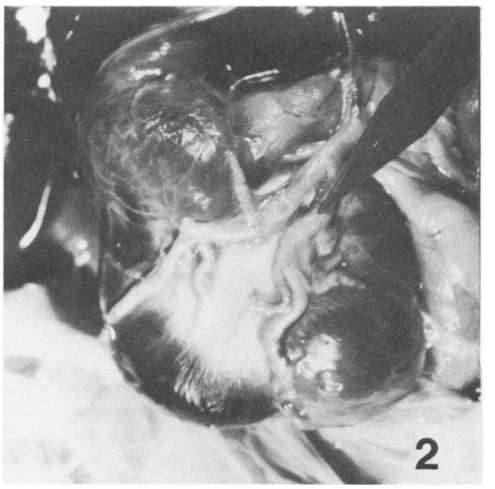
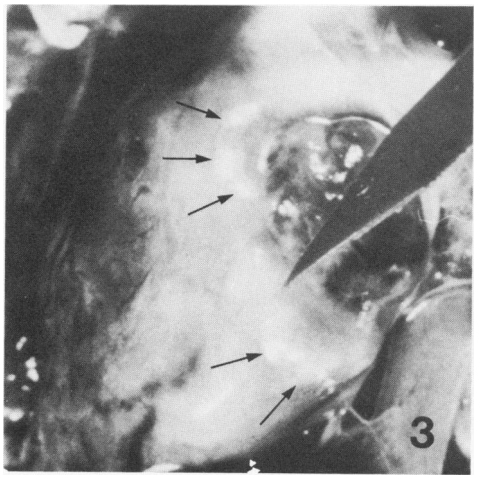






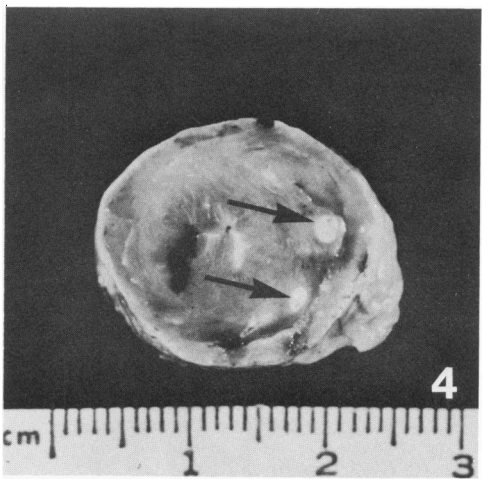
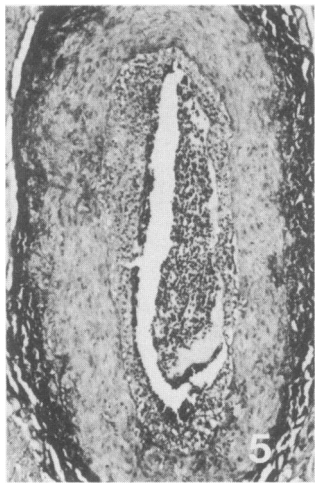
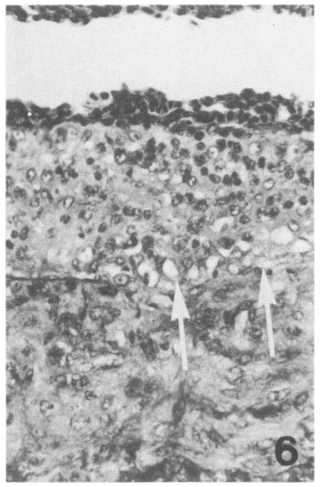
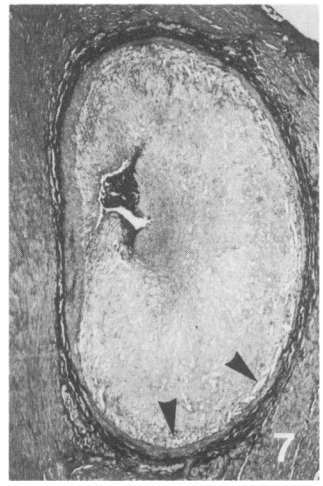
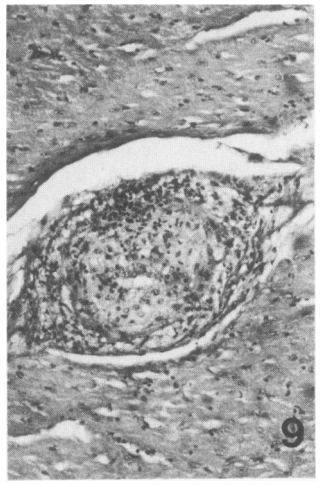


Figure 1—Gizzard and gastric arteries of uninfected chicken fed cholesterol-poor diet (Group III). Gastric arteries reveal no grossly visible atherosclerosis (*large arrow*). Grossly visible atherosclerosis was never seen in gastric arteries or other major aortic branches of normocholesterolemic or hypercholesterolemic uninfected chickens (Groups III and IV). (×2) **Figure 2**—Gizzard and gastric arteries of chicken infected with Marek's disease herpesvirus (MDV) and fed cholesterol-supplemented diet for 15 weeks (Group II). Note grossly obvious atherosclerotic change involving nearly the entire length of the left gastric artery and its branches (*large arrow*). Microscopic change in similarly involved gastric and mesenteric arteris is illustrated in Figures 11 through 16. Similar atherosclerotic change was seen in gastric arteries and other major aortic branches of several normocholesterolemic and hypercholesterolemic chickens infected with MDV (Groups I and II). Atherosclerotic change was not seen in gastric and mesenteric arteries of hypercholesterolemic and normocholesterolemic uninfected controls (Groups III and IV) (Figure 1). (×2) **Figure 3**—Heart and adjacent thoracic cavity of chicken illustrated in Figure 2. Note grossly visible atherosclerotic change in the subendocardial coronary arteries (*small arrows*). (×2) **Figure 4**—Cross section of heart illustrated in Figure 3. The lumens of major coronary arteries are occluded by atherosclerotic change (*arrows*). Grossly visible coronary artery lesions were seen in several hypercholesterolemic chickens infected with virus and fed cholesterol-supplemented diet. Surprisingly, these arterial lesions were also found in virus-infected normocholesterolemic chickens fed diets low in cholesterol. Such change was never seen in large coronary arteries of uninfected chickens. (×1.85) (All with a photographic reduction of 9%)









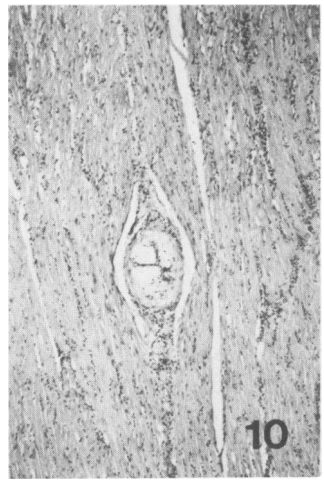


Figure 5—Major coronary artery of normocholesterolemic Group I chicken. Intima is eccentrically thickened by fibrocellular, predominantly proliferative change. (Weigert-van Gieson, × 90) **Figure 6**—Higher magnification of intima of major coronary artery illustrated in Figure 5. Note fibrocellular intimal thickening and vacuolated lipid-containing cells deep in the intima adjacent to the focally disrupted internal elastic lamina (*arrows*). The smooth-muscle cells in the subjacent media have lost their normal orientation, and occasional vacuolated cells are present. (Weigert-van Gieson, × 240) **Figure 7**—Major coronary artery of Group II chicken. Arterial lumen is nearly occluded by atheromatous intimal change. Cholesterol clefts are present in atheromatous intima adjacent to the media (*arrowheads*). The subjacent media is markedly thinned. There is no cellular reaction in adventitia and little adventitial thickening. (Weigert-van Gieson, × 30) **Figure 8**—Higher magnification of the right lateral portion of coronary artery illustrated in Figure 7. Note fibrocellular cap overlying atheromatous change deep in the intima. Atheromatous change is characterized by large quantities of extracellular lipid and vacuolated lipid-containing foam cells. (Weigert-van Gieson, × 90) **Figure 9**—Medium-sized coronary artery in myocardium of Group II chicken. Note occlusive fatty-proliferative intimal thickening and medial change with adventitial infiltrate. (H&E, × 160) **Figure 10**—Fatty, relatively acellular lesion of small coronary artery typical of those seen in Group IV chickens fed cholesterol supplement but not infected with virus. The majority of arterial lesions in this group were fatty and involved small and medium-sized myocardial arteries. (H&E, × 60) (All with a photographic reduction of 16%)

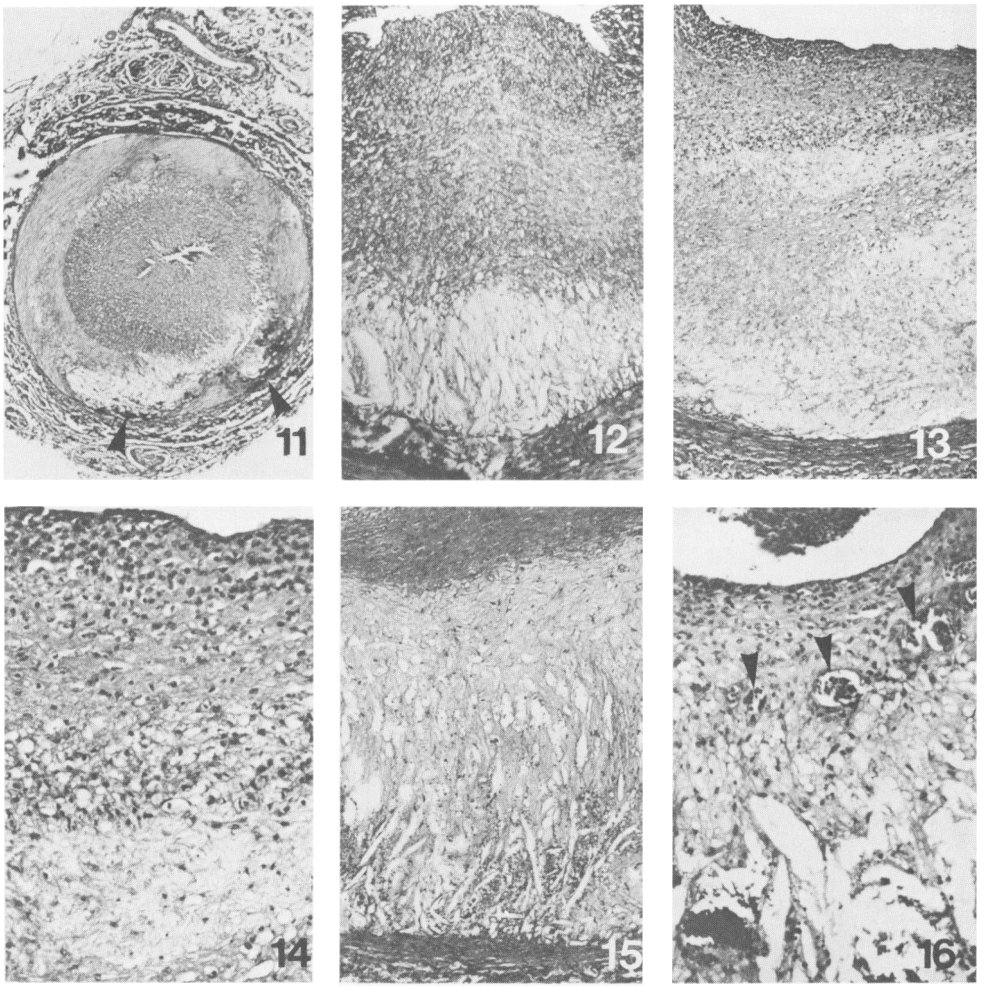
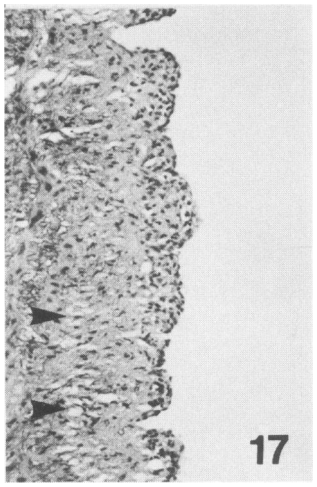
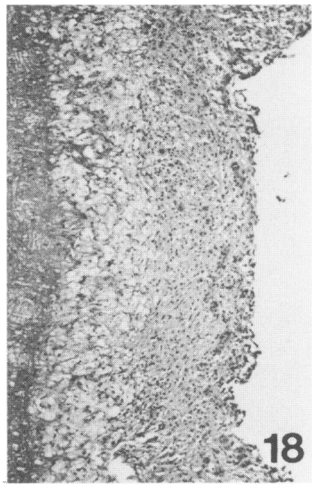
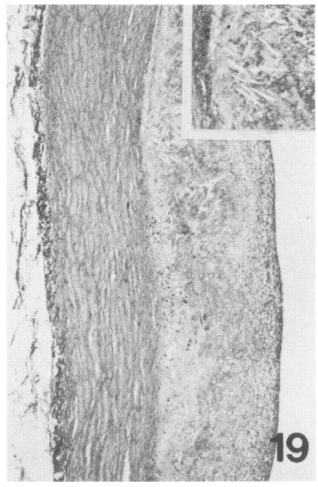
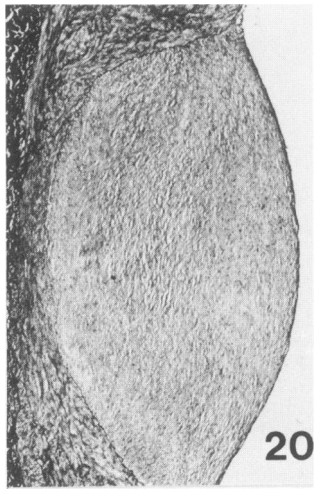


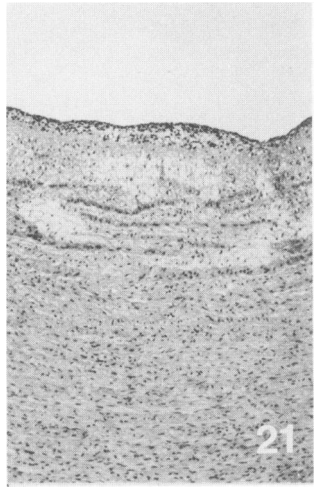
Figure 11—Gastric artery of normocholesterolemic chicken infected with MDV and fed diet low in cholesterol (Group I). Lumen of artery is occluded by thickened intima. Note atheromatous change deep in intima and media. Media adjacent to atheromatous change is focally calcified (*arrowheads*). This degree of atherosclerotic change was present in celiac, gastric, and mesenteric arteries of several Group I chickens. (Weigert-van Gieson, × 39) **Figure 12**—Serial section of artery illustrated in Figure 11. Fibrocellular intimal cap covers pool of extracellular lipid deep in the intima. Underlying media is markedly thinned and calcified. (Weigert-van Gieson, × 95) **Figure 13**—Atheromatous change in mesenteric artery of Group II chicken. Note lipid deposit deep in intima and markedly thinned media. Similar lesions were seen in gastric, mesenteric, and celiac arteries of several chickens in Groups I and II. Lesions in these arterial beds were not seen in chickens of Groups III and IV. (H&E, × 65) **Figure 14**—Higher magnification of serial section of intima of artery illustrated in Figure 13. Atheromatous change characterized by foam cells and extracellular lipid is present in the deeper portion of the intima. Note overlying fibrocellular cap. (H&E, × 170) **Figure 15**—Higher magnification of atheromatous change in mesenteric artery of Group II chicken. Note atheromatous gruel characterized by large numbers of cholesterol clefts, calcium deposits, and cellular debris. (H&E, × 205) **Figure 16**—Intimal fibrocellular cap and underlying atheromatous change in segment of artery adjacent to that illustrated in Figure 15. Note vascularization of intimal cap (*arrowheads*). Similar vascularization of intimal lesions was often present in chronic arterial lesions of virus-infected chickens. (H&E, × 150) (All with a photographic reduction of 16%)











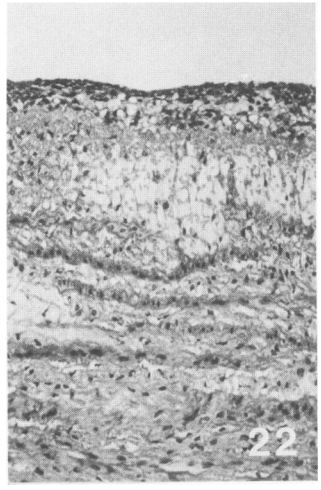
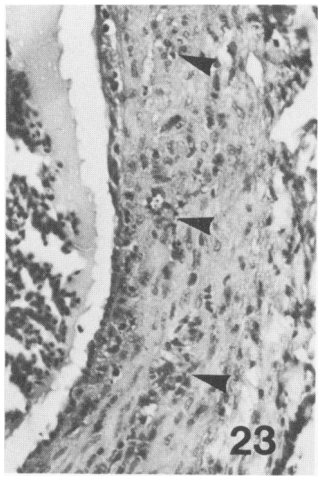
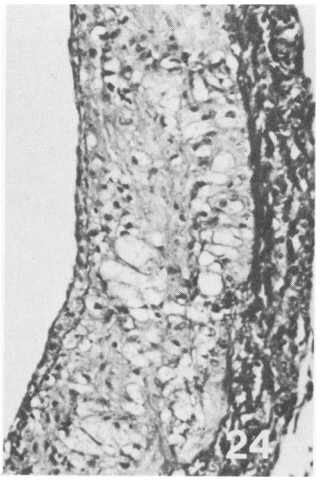
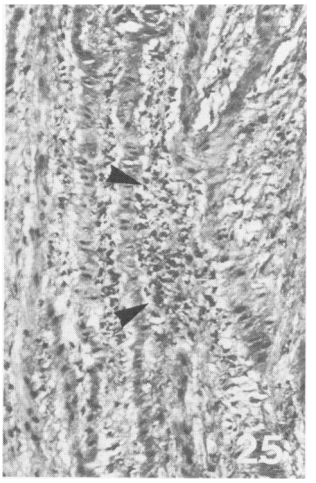
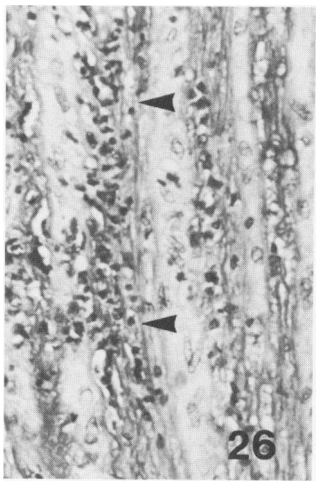


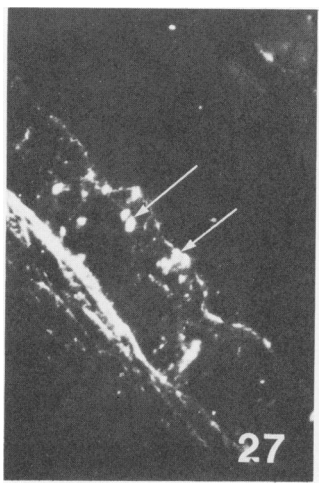
Figure 17—Fatty-proliferative intimal thickening in proximal descending aorta of Group I chicken. Note clusters of foam cells deep in the intima. (*arrowheads*). (H&E, × 150) **Figure 18**—Fatty-proliferative intimal thickening in proximal portion of descending aorta of Group II chicken. Note large numbers of lipid-laden foam cells deep in the intima. Compare thickness of intima and the amount of fatty change with that of Group I chicken illustrated in Figure 17. Intima in Group II chickens was thicker and contained more lipid than that in Group I. (H&E, × 75) **Figure 19**—Atheromatous change in intima of mid-portion of descending proximal aorta of Group II chicken. Note cholesterol clefts deep in intima, shown in higher magnification in **inset.** (H&E, × 30; **inset,** × 50) **Figure 20**—Arteriosclerotic plaque in distal aorta of Group I chicken. In contrast to the lesions in the proximal aorta that were found exclusively in chickens infected with MDV, lesions in the distal aorta were found as commonly in uninfected chickens. (H&E, × 55) **Figure 21**—Fatty-proliferative thickening in intima of proximal aorta of Group II chicken. Intimal thickening overlies area of medial degeneration containing foam cells and extracellular lipid. (H&E, × 70) **Figure 22**—Higher magnification of aortic lesion illustrated in Figure 21. Note partial loss of normal architectural pattern of aorta in the media beneath fatty proliferative intimal plaque. In aortas and muscular arteries of many virus-infected chickens, such effacement of media appeared to contribute to intimal thickening. (See Figure 11.) (H&E, × 150) (All with a photographic reduction of 16%)











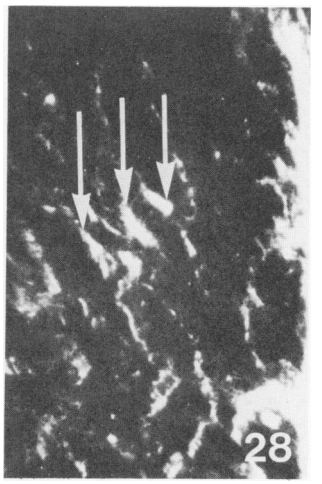
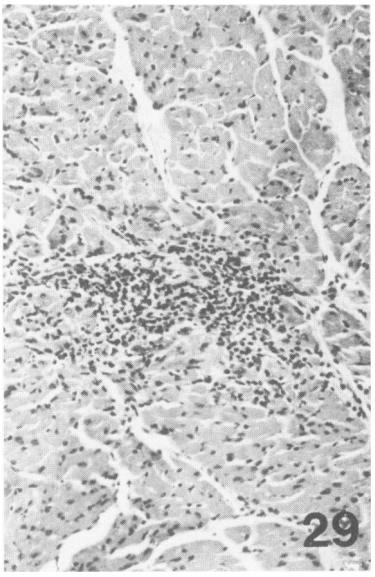
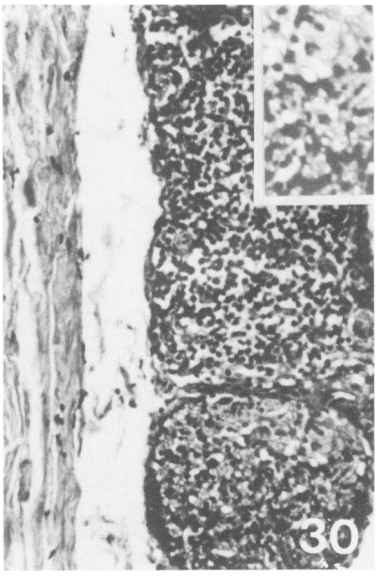
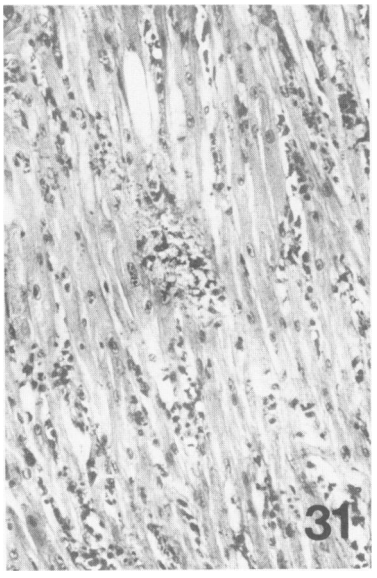


Figure 23—Major coronary artery of Group I chicken. Note focal medial necrosis associated with lymphocytic infiltrate in arterial lesion without intimal thickening (*arrowheads*). Similar foci of medial necrosis were also found in association with areas of intimal thickening. Similar medial lesions were seen in chickens that died a few weeks after infection. (H&E, × 220) **Figure 24**—Major coronary artery of Group II chicken that survived for 7 months of experiment. Foci of medial necrosis, similar to those seen in Figure 23, were associated with accumulation of lipid-laden foam cells. (H&E, × 340) **Figure 25**—Media of aorta of infected chicken fed a cholesterol-supplemented diet. Chicken died a few weeks after infection with MDV.[101] Scattered foci of lymphocytic infiltration and medial necrosis are present (*arrowheads*). There was no intimal change. Similar change was seen in the media of aortas of Group I and II chickens killed 7 months after infection. In some instances there was intimal thickening. (H&E, × 155) **Figure 26**—Aorta of chicken infected with MDV and fed cholesterol-poor diet. Chicken died a few weeks after infection with virus. Note extensive area of medial necrosis and infiltration with lymphocytes (*arrowheads*). No appreciable intimal change was present. Similar change was seen in media of aortas of Group I chickens killed 7 months after infection. In some instances there was intimal thickening. (H&E, × 330) **Figure 27**—Gastric artery with atheromatous change from a 7-month-old Group II chicken. The frozen longitudinal section of artery was stained with specific antibody to MDV conjugated with fluorescein isothiocyanate. Note cells in media of artery with diffuse cytoplasmic fluorescence for Marek's herpes antigen (*arrows*). (× 80) **Figure 28**—Higher magnification of gastric artery illustrated in Figure 27. Specific cytoplasmic fluorescence for Marek's antigen is present in long thin cells that are most likely smooth-muscle cells (*arrows*). (× 320) (All with a photographic reduction of 16%)







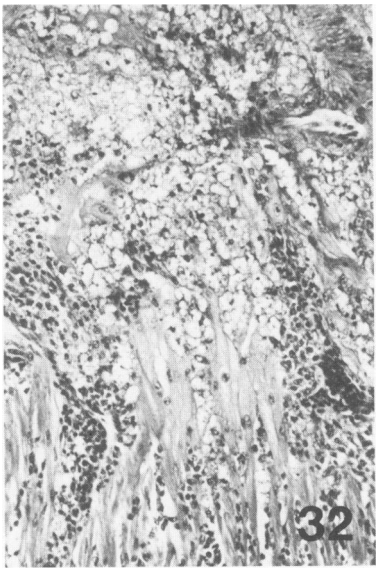


Figure 29—Infiltrate of small lymphocytes in myocardium of Group I chicken. Similar infiltrates were seen in hearts of chickens of all experimental groups but were more extensive in hearts of chickens infected with MDV. (H&E, × 150) **Figure 30**—Epicardial nerve of Group I chicken. Nerve is extensively infiltrated with lymphocytes. Similar infiltrates were seen in chickens of Group II but were never seen in chickens that were not infected with MDV. (H&E, × 150; **inset** × 335) **Figure 31**—Myocardium of Group I chicken. Myofibers are extensively infiltrated by large and intermediate-sized lymphocytes. Similar changes were seen in Group II chickens but not in chickens of Groups III and IV. (H&E, × 150) **Figure 32**—Myocardium of Group I chicken. Many large and intermediate-sized lymphocytes are present between myofibers, and extensive areas of myocytolysis and myocardial necrosis are seen. Similar necrosis was seen in hearts of Group II chickens but not in Groups III and IV. (H&E, × 155)